5 DNA QUANTITATION - YIELD GEL

5.1 GOALS:

5.1.1 To become familiar with the theories of electrophoresis as they apply to submarine gels used for DNA quantitation.

5.1.2 To learn the parameters used for electrophoresis of the yield gel.

5.1.3 To become familiar with the photographic procedures used to document yield gel results.

5.1.4 To develop an understanding and working knowledge of the use of yield gels, including interpretations, limitations, and proper documentation.

5.1.5 To become familiar with the controls run on yield gels.

5.2 TASKS:

5.2.1 Prepare reagents and gels necessary to quantitate DNA samples using a yield gel. Refer to the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedure Manual, Section III - Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 BIO System for the procedure.

5.2.2 Run yield gels.

5.2.3 Interpret the results of yield gels.

5.2.4 Look at degraded samples on a yield gel to become familiar with the appearance of a degraded sample versus a sample with high molecular weight DNA present. Note: These previously isolated samples will be provided to the training coordinator by either the Forensic Biology Section Chief or the Forensic Molecular Biologist.

5.2.5 Compare the yield gel results to the AluQuant® quantitation results obtained in Chapter 7 for the 28 blood stains isolated in Chapter 4.

5.2.6 Read applicable literature and become familiar with the glossary terms. Refer to Appendices A, B, and C.

5.2.7 Continue on to Chapter 8, NORMALIZATION WIZARD AND AMPLIFICATION PROCESSES.

5.3 TRAINING EVALUATION:

5.3.1 Knowledge

5.3.1.1 Review of notes, photographs, and worksheets in training notebook by training coordinator.

5.3.1.2 Mini-mock trials/oral and practical examination.

5.3.2 Skills

5.3.2.1 The trainee should demonstrate an unquestionably sound technique for quantitating DNA by running a sufficient number of yield gels and accurately interpreting the associated results. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

5.3.3 Completion of the trainee checklist by the training coordinator.

STUDY QUESTIONS:

1. What information about a sample can be obtained from a yield gel? What information cannot be obtained?

2. What is the purpose of lambda Hind III?

3. What calibration standards are used? How are the calibration standards made?

4. What is in loading buffer and why?

5. What percent agarose is used for the yield gel and why?

6. Why aren't acrylamide gels used to obtain better resolution?

7. How does ethidium bromide work to aid in visualizing DNA?

8. What are some problems associated with the use of ethidium bromide?

9. What would happen if ethidium bromide was left out of the gel? What would the analyst have to do?

10. Why might an analyst run a yield gel in addition to the AluQuant® Quantitation System?

11. Explain the appearance of degraded vs. non-degraded DNA on a yield gel.

CHECKLIST FOR DNA QUANTITATION – YIELD GEL

Name of Trainee: _____

1. Trainee has prepared reagents and gels necessary to quantitate DNA samples using a yield gel.

   Date:_____ Training Coordinator:_____

   Comments:_____

2. Trainee has successfully and accurately completed all appropriate paperwork associated with the yield gel.

   Date:_____ Training Coordinator:_____

   Comments:_____

3. Trainee has successfully run yield gels for all required samples extracted as addressed in chapter 4, DNA Isolation.

   Date:_____ Training Coordinator:_____

   Comments:_____

4. Trainee has successfully interpreted the results for all yield gels.

   Date:_____ Training Coordinator:_____

   Comments:_____

5. Trainee has evaluated the appearance of degraded DNA when run on a yield gel.

   Date:_____ Training Coordinator:_____

   Comments:_____

6. Trainee has developed a basic understanding of the advantages and limitations associated with the yield gel and how these results compare when the same samples were run using the AluQuant® Quantitation System.

   Date:_____ Training Coordinator:_____

   Comments:_____

7. Notebook is organized and complete.

   Date:_____ Training Coordinator:_____

   Comments:_____

8. Trainee has participated in mini-mock trials and/or question and answer sessions.

Date:_____ Training Coordinator:_____

Comments:_____

♦**END**